

Immunocompetence of Juvenile Chinook Salmon Following Exposure To Dietary PCBs: Implications for Regulatory Policy

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Abstract

Protection of threatened Puget Sound chinook salmon is a goal we all share. With ProFishent, Inc., we conducted experiments to investigate whether PCBs in the exposure regime of the Lower Duwamish Waterway may cause immunosuppression effects among outmigrating chinook salmon. The results of these experiments are discussed in a parallel presentation.

The objective of this talk is to evaluate the implications of these results from a regulatory perspective. With the listing of Puget Sound chinook salmon under the Endangered Species Act (ESA), take of individuals is prohibited. To evaluate risk of harm, endpoints in addition to the traditional suite of growth, survival, and reproduction are attracting more attention and should be critically evaluated before use in regulatory programs. Some studies have suggested that immunosuppression impacts are possible as juvenile chinook migrate through the Lower Duwamish Waterway. Although the specific causative chemicals and threshold concentrations have not been identified, PCBs and PAH compounds have been mentioned as potential candidates. Results suggest that total PCB concentrations of 18 ppm dw in food, resulting in body burdens of approximately 1 ppm ww in fish, do not cause immunosuppression in juvenile chinook salmon. We will discuss the implications for Superfund, Natural Resource Damage Assessment, and ESA.

Introduction

Outmigrating juvenile chinook salmon use the Lower Duwamish Waterway (LDW) for migration and early developmental growth. These fish are listed as threatened under the Endangered Species Act and the Lower Duwamish Waterway has been listed as a Superfund site. It is therefore of interest to determine whether dietary exposure to polychlorinated biphenyls (PCBs) at concentrations similar to those in the LDW affects survival, growth, and immunocompetence of juvenile chinook salmon. These data will be useful in ecological risk assessments for the RI, in ESA consultations, and potentially in NRDA claims.

The study examined the ability of juvenile chinook to respond to the bacterial pathogen *Vibrio anguillarum* following a 28-day dietary exposure to Aroclor 1254. Both innate and adaptive immunocompetence were assessed, using susceptibility to *in vivo* challenge with the fish pathogen *V. anguillarum* as the indicator. A parallel study was also conducted to test the effects of juvenile chinook salmon size and water salinity on their ability to withstand controlled laboratory exposure to this pathogen. These two studies were conducted in parallel by ProFishent Inc. at the Battelle Marine Sciences Laboratory using juvenile chinook salmon from Voights Creek Hatchery in Orting, Washington. Only the results from the innate immunocompetence of the PCB study were presented in the talk. Methods and results from the adaptive immunocompetence of the PCB study, as well as the methods and results from the size and salinity study, were presented in a companion poster (Powell and others 2000) and are in preparation for publication in peer-review journals at this time.

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survival, and reproduction are attracting more attention and should be critically evaluated before use in regulatory programs. Some studies have suggested that immunosuppression impacts are possible as juvenile chinook migrate through the Lower Duwamish Waterway. Although the specific causative chemicals and threshold concentrations have not been identified, PCBs and PAH compounds have been mentioned as potential candidates.

Concentrations for the three PCB treatment levels used in this study were determined to be inclusive of the range of probable concentrations in prey species of juvenile chinook salmon from the LDW, and to provide a geometric increase in dose to establish whether a dose-response relationship exists between PCB concentration and immunocompetence (Windward 2000). Nominal concentrations were as follows: control, 0.32, 1.0, 3.0, and 10 mg Aroclor 1254/kg-food (wet weight). The control has no added PCBs, but the Moore-Clark salmon feed contained low background concentrations of PCBs. The low dose level (0.32mg Aroclor 1254/kg-food) is believed to be the best estimate of the dose from prey items in the LDW (Windward 2000).

Methods

Five-month-old juvenile fish weighing approximately 3 g each were collected April 27, 2000 from the Washington Department of Fish and Wildlife Voights Creek Hatchery and brought to Battelle for a five-week acclimation period prior to testing.

Immunocompetence was tested using five groups of juvenile chinook salmon: three PCB treatment groups that were exposed to three treatment levels of PCB Aroclor 1254 administered orally through their diet; a *Vibrio* positive control that was not exposed to PCBs but was exposed to *V. anguillarum*, and a negative control that received neither PCB nor *Vibrio* treatments. Subsequent to PCB exposure, PCB treatment groups and the *Vibrio* positive control were transferred to five replicate *V. anguillarum* challenge tanks and exposed to an LC60 concentration of *V. anguillarum*. The negative control was transferred to five replicate challenge tanks but was not exposed to *V. anguillarum*. The highest PCB dose was tested for palatability. Weight and length measurements were taken when fish first arrived at the laboratory, prior to and just after the 28 day exposure of PCB-spike food, and prior to and one week after the *V. anguillarum* challenge. Fish were collected for tissue samples at the same times during the study, except for the initial sampling period (i.e., upon arrival to the laboratory).

Upon completion of the first set of challenges, *V. anguillarum* challenge tanks used in the first set were cleaned for use in *Vibrio* challenges in the second set. After completion of the *V. anguillarum* challenge with the first set of fish, a second set of fish was vaccinated with *V. anguillarum*. These fish were orally dosed with PCBs along with the first set of fish, but subsequently fed on clean salmon feed for 24 days. Following vaccination, these fish were exposed to *V. anguillarum* and their mortality was monitored for 14 days. The methods and results from this test were not presented in the talk, but were discussed in the companion poster at the PSRC (Powell and others 2001) and are being described in detail in a manuscript being prepared for submittal to peer-reviewed journals this spring.

Fourteen-day cumulative mortality was compared among the treatment levels, with tank as the experimental unit. Results from set 1 were analyzed separately from those of set 2. The untreated negative controls (fish exposed to neither PCBs nor *V. anguillarum*) were included for quality assurance purposes only, and were not used for statistical analyses. The results were statistically analyzed using a one-way ANOVA with $p \leq 0.05$. Percent cumulative mortality data were normalized using an arcsine square-root transformation. Fisher's least-significant-difference tests were run where the ANOVA rejected a multisample hypothesis of equal means. Growth data were compared among the various PCB oral exposure treatments and controls following the 28-day PCB exposure and four weeks later. Mortality within the exposure tanks was continuously monitored from the start of PCB exposure through the duration of the study.

Results

To avoid restrictions in publishing the data, results from the studies will be described in this section, but actual results will not be presented. All of the results from the studies will be submitted to peer-review journals this year and a gray literature document will also be available this year with all of the raw data.

In whole body fish tissue, PCB Aroclors, lipids and total solids were measured at ARI. Composite samples analyzed were collected at 4, 5, 8, and 11 weeks from the initiation of the study. Fish were fed Aroclor 1254-spiked feed for the initial 28 days of the study, followed by unspiked feed for the duration of the study, except during the challenge period (weeks 6 and 7) when the fish were not fed. Concentrations in measured in the fish (0.04 to 1.0 mg/kg ww) bracketed those reported for field-collected fish. NMFS 2000 (unpublished data) reports average and maximum concentrations of 0.039 and 0.10 mg/kg ww in wild juvenile chinook salmon collected near Kellogg Island and 0.22 and 0.80 mg/kg ww in hatchery juvenile chinook salmon collected in Slip 4. Juvenile salmonids were also collected for analysis last summer as part of this program. These fish have just been submitted for analysis of PCB Aroclors and congeners.

An insignificant number of fish died in the dosing tanks or in the control tanks during the first 28 days of the experiment. Also, no mortalities were observed within 96 hours following oral gavage of 6 PCB concentrations. Body burdens in these orally dosed fish were up to 60,000 ppb ww Aroclor 1254. Because no mortalities occurred, an LC50 for Aroclor 1254 could not be calculated. No effects from PCBs were observed on fish weight or length at weeks 4 or 8 during the study.

Following exposure to *V. anguillarum*, classic response curves were observed with increasing cumulative mortality as a function of time over the 14-day interval. Most of the observed mortality occurred between days 4 and 7. A one-way ANOVA conducted with day 14 data showed no significant differences among the four treatments including the challenged control and the low, mid, and high-dosed fish. Low variance was observed among the treatments. Nearly 100 percent of the mortalities were confirmed to be due to *Vibrio*. The unchallenged controls showed very low mortality (less than 4 percent).

Discussion

The data from this study will be useful in three programs involving chinook salmon and the Lower Duwamish Waterway: Superfund, Endangered Species Act (ESA), and Natural Resource Damage Assessments (NRDA). In the Remedial Investigation ecological risk assessment (ERA) for the Lower Duwamish Waterway, these data will be included in the effects and exposure assessments. This ERA is currently underway and is in the Problem Formulation stage. For ESA, these data will be useful for Section 7 consultations as well as in the matrix of pathways and indicators used in effect determinations. For NRDA, the data will be useful in ongoing and potential NRDA sites in the Northwest where salmon are listed. Single-contaminant studies are important considerations in causality and injury assessments.

Chinook salmon have a complex life history and to ultimately restore salmon it will be necessary to examine stressors in all life stages. From a relative risk perspective, it is important to examine all stressors within a watershed and over the home range of the species. This work is one piece of the puzzle, examining a potential chemical stressor (PCBs) and the smolt/parr stage that uses the Superfund site area (LDW). Through the use of carefully controlled laboratory studies, key variables can be isolated to address uncertainty in critical areas. In combination with field data, resource management decisions can be made to best restore the salmon population.

Literature cited

- Powell, D.P., R.C. Palm, A.D. Skillman, K.L. Godtfredsen, and D. Hotchkiss. 2001. Immunocompetence of juvenile chinook salmon as a function of fish size and exposure to dietary polychlorinated biphenyls. Puget Sound Research 2001. Poster Presentation.
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